BIOKINETICS AND EFFECTS OF NANOPARTICLES[1]

GUNTER OBERDÖRSTER[*]
*University of Rochester School of Medicine and Dentistry,
Department of Environmental Medicine, 575 Elmwood Avenue,
MRBx Building, Box 850, Rochester, New York 14642, USA*

Abstract: Exposures to airborne nanosized particles (<100 nm) have been experienced by humans throughout their evolutionary stages. Recently, the rapidly developing field of nanotechnology is likely to become yet another source for human exposures to nanosized particles – engineered nanoparticles (NPs) – by different routes, i.e., inhalation, ingestion, dermal, or even injection. Nanotechnology is defined as research and technology development at the atomic, molecular, or macromolecular levels, in the length scale of ~1–100 nm range. One of the many promising applications of engineered NPs is in the area of medicine, for example, targeted drug delivery as aerosols and to tissues which are difficult to reach. The discipline of nanomedicine has arisen to develop, test, and optimize these applications. However, the same properties that makes NP attractive for development in nanomedicine and for specific industrial processes could also prove deleterious when NP interact with cells. An emerging discipline – nanotoxicology, which can be defined as safety evaluation of engineered nanostructures and nanodevices – is gaining increased attention. Nanotoxicology research will not only provide information for risk assessment of NP based on data for hazard identification, dose–response relationships, and biokinetics, but will also help to advance further the field of nanomedicine by providing information to alter undesirable NP properties. Although potential adverse effects of engineered NP have not been systematically investigated, there are a number of studies in the area of inhalation toxicology and also human epidemiology from which some preliminary conclusions about effects of nanosized particles can be drawn. There are also some decades-old – mostly forgotten – studies with nanosized particles which shed light on the biokinetics of such particles once introduced into the organism. This presentation summarizes results of studies with nanosized particles with a focus on the respiratory tract and skin as portals of entry. Examples of translocation and effects of nanosized particles and presumed

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[*] To whom correspondence should be addressed.

[1] This presentation consists for the most part of updated excerpts of a review by Oberdörster et al., 2005, *Environ. Health Perspect*. 113: 823–839.

mechanisms will be highlighted. They illustrate, on the one hand, that we need to be aware of possible acute adverse effects and potential long-term consequences; on the other hand, the findings also give us ideas about the intriguing possibilities that NP offer for potential use as diagnostic tools or as therapeutic delivery systems. A thorough evaluation of desirable versus adverse effects is required for the safe use of engineered NP, and major challenges lie ahead to answer key questions of nanotoxicology, foremost being the assessment of human and environmental exposure, the identification of potential hazards (toxicity *vs*. benefit), and the biopersistence in cells and subcellular structures. Results so far demonstrate that the highly desirable properties of nanoparticles, which makes them attractive as medicinal aerosols, as well as their potential to induce toxicity, depend not only on their size but on a variety of surface properties. To establish the principles which govern NP-cell interactions will be a major challenge for the field of Nanotoxicology.

Keywords: nanoparticle, toxicity, risk, respiratory tract, skin, translocation

1. Introduction

Exposures to airborne ultrafine particles (UFPs, <100 nm) have been experienced by humans throughout their evolutionary stages, but it is only with the advent of the industrial revolution that such exposures have increased dramatically because of anthropogenic sources such as internal combustion engines, power plants, and many other sources of thermodegradation. The rapidly developing field of nanotechnology is likely to become yet another source for human exposures to engineered nanoparticles (NPs) by different routes: inhalation (respiratory tract), ingestion gastrointestinal GI tract, dermal (skin), and injection (blood circulation). Table 1 summarizes some of the natural and anthropogenic sources of NPs, the latter divided into unintentional and intentional sources.

   Obvious differences between unintentional and intentional anthropogenic UFP are the polydispersed and chemically complex nature (elemental, soluble, and volatile carbon compounds; soluble and poorly soluble inorganics) (Cyrys et al. 2003; Hughes et al. 1998) of the former, in contrast to the monodisperse and precise chemically engineered characteristics and solid form of the latter, generated in gas or liquid phase (National Nanotechnology Initiative [NNI] 2004). However, despite these differences, the same toxicologic principles are likely to apply for NPs, because not only size but also a number of other particle parameters determine their biologic activity (Table 2).

TABLE 1. Ultrafine/nano Particles (<100nm): natural and anthropogenic sources.

Natural	Anthropogenic	
	Unintentional	*Intentional*
Gas to particle conversions	Internal combustion engines	Engineered nanoparticles:
Forest fires	Power plants	*(controlled size and shape,*
Volcanoes *(hot lava)*	Incinerators	*designed for functionality)*
Viruses	Airplane jets	metals, semiconductors,
Biogenic magnetite:	Metal fumes	*metal oxides*
magnetotactic bacteria;	*(smelting, welding, etc.)*	*quantum dots/rods*
protictists, mollusks,	Polymer fumes	*fullerenes, nanotubes*
arthropods, fish,	Other fumes	*nanowires*
birds, human brain,	Heated surfaces	*nanoshells*
meteorite?	Frying, boiling, grilling	*nanorings…etc.*
Ferritin (12.5 nm)	Electric motors	*(nanotechnology applied to*
Microparticles *(<100nm)*		*many products: cosmetics,*
(activated cells)		*medical, fabrics, electronics,*
		optics, displays, etc.)
	Human Exposure Routes	
	Ultrafine particles:	
	Inhalation	
	Nanoparticles: *Inhalation*	
	Ingestion Dermal Injection	

Figure 1 depicts the range of sizes of airborne ambient particulate matter, including the nucleation-mode, Aitken-mode, accumulation- mode, and coarse-mode particles. Ambient particles <0.1 μm, defined as UFPs in the toxicologic literature, consist of transient nuclei or Aitken nuclei (NRC 1983). More recently, even smaller particles in the nucleation mode with peak diameters around 4 nm have been observed (McMurry and Woo 2002).

TABLE 2. Ultrafine Particles and Engineered Nanoparticles (non-fibrous)- Interchangeable Terms?

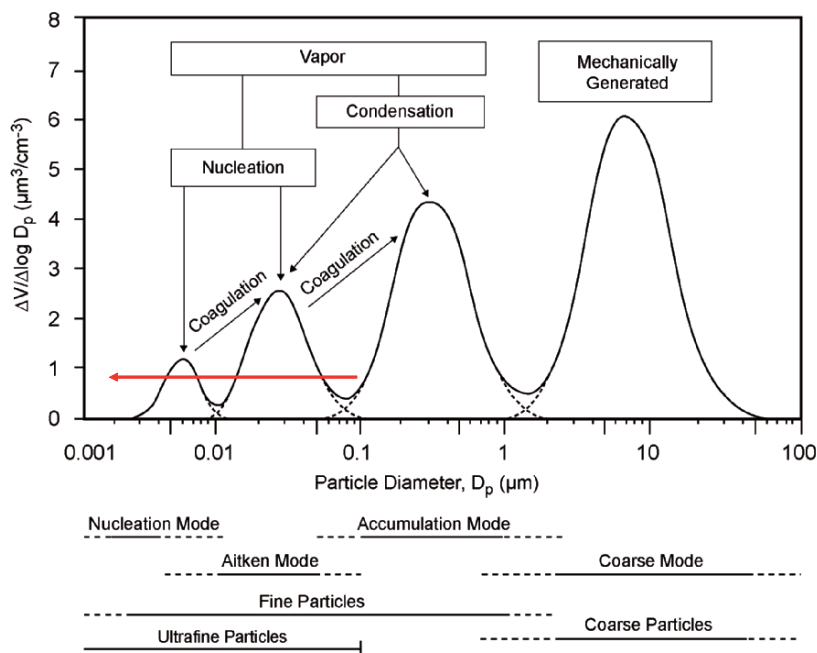| Both Consist of nanosized particles:	Ultrafine Particles	Engineered NP
Primary particles		
– Size	<100 nm	<100 nm
– Size distribution	Polydispersed	Monodispersed
Aggregations when generated	Yes	No
Agglomeration in air	Yes	Yes
Chemistry	variable to well defined	well defined
Toxicological significance	small size, high surface areas per mass, chemistry	

Figure 1. Idealized size distribution of traffic-related particulate matter. The four polydisperse modes of traffic-related ambient particulate matter span approximately 4 orders of magnitude from below 1 nm to above 10 μm. Nucleation and Aitken mode particles are defined as ultrafine particles (less than ~100 nm). Source-dependent chemical composition is not well controlled and varies considerably. In contrast engineered nanoparticles (1–100 nm) have well-controlled chemistry and are generally monodispersed (From US EPA 2004.)

Table 3 shows the tremendous differences in particle number concentrations and particle surface areas for particles of the four ambient modes, assuming an airborne concentration of 10 pg/cm^3 of unit density particles of each size. The extraordinarily high number concentrations of NPs per given mass will likely be of toxicologic significance when these particles interact with cells and subcellular components. Likewise, their increased surface area per unit mass can be toxicologically important if other characteristics such as surface chemistry and bulk chemistry are the same. Although the mass of UFPs in ambient air is very low, approaching only 0.5–2 μg/m^3 at background levels (Hughes et al. 1998), it can increase several fold during high-pollution episodes or on highways (Brand et al. 1991; Shi et al. 2001; Zhu et al. 2002).

TABLE 3. Particle Number and Particle Surface Area per 10 pg/cm^3 Airborne Particles of Unit Density.

Particle diameter (nm)	Particle number (N/cm^3)	Particle surface area (μm^3)
5	153,000,000	12,000
20	2,400,000	3,016
250	1,200	240
5,000	0.15	12

Small size, high number per mass, and surface chemistry confer both desirable and undesirable properties.

Detailed Physico-chemical characterization of NP is essential.

1.1. PHYSICOCHEMICAL CHARACTERISTICS AS DETERMINANTS OF BIOLOGIC ACTIVITY

The small size and corresponding large specific surface area of solid NPs confer specific properties to them, for example, making them desirable as catalysts for chemical reactions. The importance of surface area becomes evident when considering that surface atoms or molecules play a dominant role in determining bulk properties (Amato 1989); the ratio of surface to total atoms or molecules increases exponentially with decreasing particle size. Increased surface reactivity predicts that NPs exhibit greater biologic activity per given mass compared with larger particles, should they be taken up into living organisms and provided they are solid rather than solute particles. This increased biologic activity can be either positive and desirable (e.g., antioxidant activity, carrier capacity for therapeutics, penetration of cellular barriers for drug delivery) or negative and undesirable (e.g., toxicity, induction of oxidative stress or of cellular dysfunction), or a mix of both.

The characteristic biokinetic behaviors of NPs are attractive qualities for promising applications in medicine as diagnostic and therapeutic devices and as tools to investigate and understand molecular processes and structures in living cells (Akerman et al. 2002; Foley et al. 2002; Kreuter 2001; Li et al. 2003). For example, targeted drug delivery to tissues that are difficult to reach (e.g., central nervous system [CNS]), NPs for the fight against cancer, intravascular nanosensor, and nanorobotic devices, and diagnostic and imaging procedures are presently under development. The discipline of nanomedicine – defined as medical application of nanotechnology and related research – has arisen to design, test, and optimize these applications so that they can eventually be used routinely by physicians. There are also many promising applications in different industrial fields in addition to nanomedicine, promising a bright future for nanotechnology (Fig. 2).
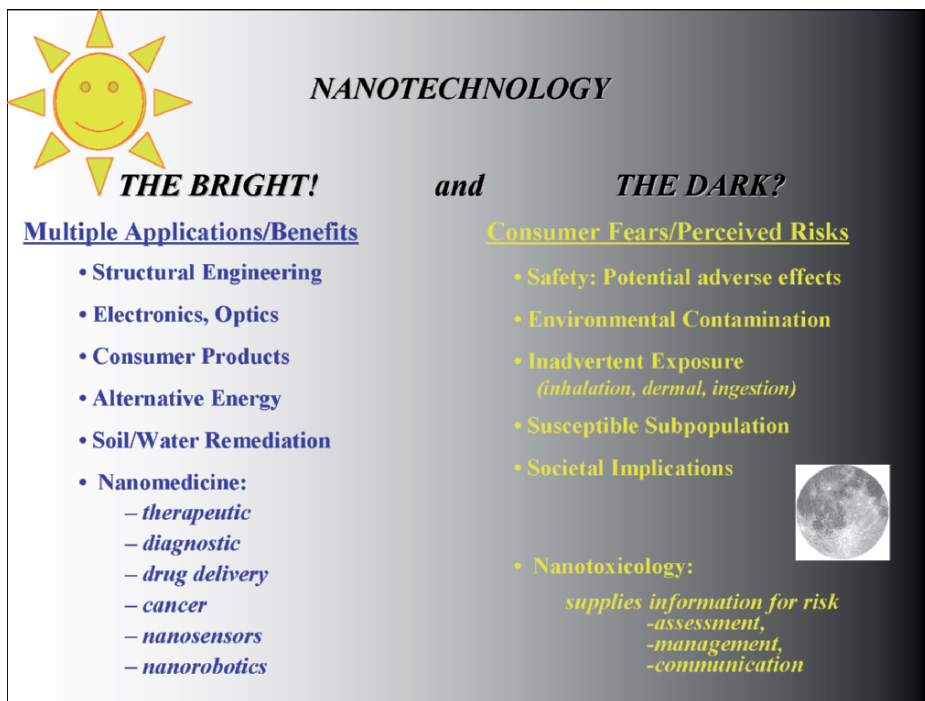
Figure 2. The many promising uses of nanotechnology may be compromised by the potential to cause adverse effects. Appropriate toxicological testing is necessary to determine a potential risk.

However, in apparent stark contrast to the many efforts aimed at exploiting desirable properties of NPs for multiple industrial applications and for improving human health are the limited attempts to evaluate potential undesirable effects of NPs when administered intentionally for medicinal purposes, or after unintentional exposure during manufacture or processing for industrial applications. The same properties that make NPs so attractive for development in nanomedicine and for specific industrial processes could also prove deleterious when NPs interact with cells, reflecting a potentially dark side of nanotechnology (Fig. 2). Thus, evaluating the safety of NPs should be of highest priority given their expected worldwide distribution for industrial applications and the likelihood of human exposure, directly or through release into the environment (air, water, soil). Nanotoxicology – "science of engineered nanodevices and nanostructures that deals with their effects in living organisms" – is gaining increased attention. Nanotoxicology research not only will provide data for safety evaluation of engineered nanostructures and devices

but also will help to advance the field of nanomedicine by providing information about their undesirable properties and means to avoid them.

1.1.1. Concepts of Nanotoxicology

Studies with ultrafine and fine titanium dioxide (TiO_2) particles showed that ultrafine anatase TiO_2 (20 nm), when instilled intratracheally into rats and mice, induced a much greater pulmonary-inflammatory neutrophil response (determined by lung lavage 24 h after dosing) than did fine anatase TiO_2 (250 nm) nm) when both types of particles were instilled at the same mass dose (Fig. 3a). Also, expressed as particle number showed significant differences in the inflammatory response. However, when the instilled dose was expressed as particle surface area, it became obvious that the neutrophil response in the lung for both ultrafine and fine TiO_2 fitted the same dose response curve (Fig. 3b), suggesting that particle surface area for particles of different sizes but of the same chemistry and crystallinity, is a better dosemetric than is particle mass or
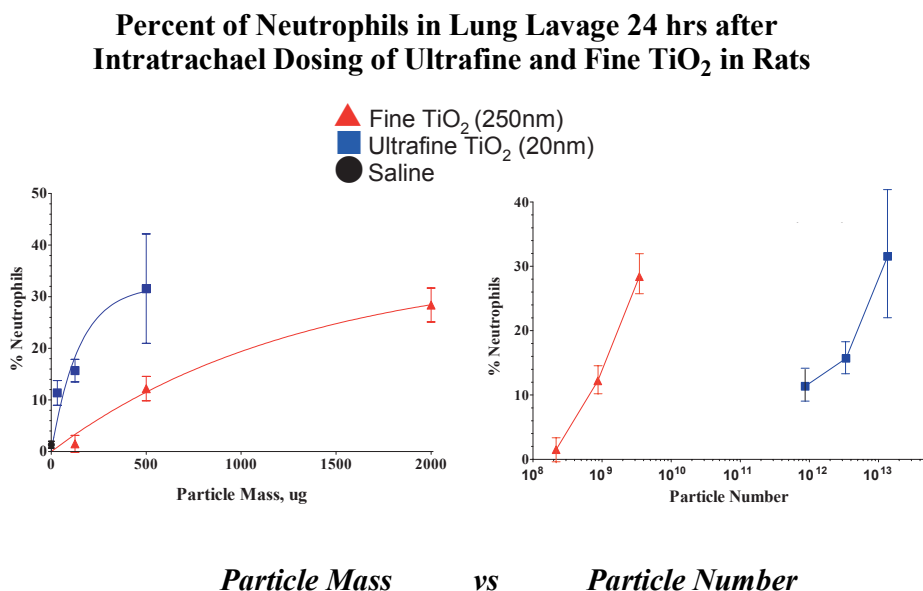

Figure 3a. Percent of neutrophils in lung lavage of rats as indicators of inflammation 24 h after intratracheal instillation of different mass doses of 20 and 250 nm TiO_2 particles in rats and mice. The steeper dose–response of nanosized TiO_2 is obvious when dose is expressed as mass or particle number.

Percent of Neutrophils in BAL 24 hrs after Instillation of TiO$_2$ in Rats
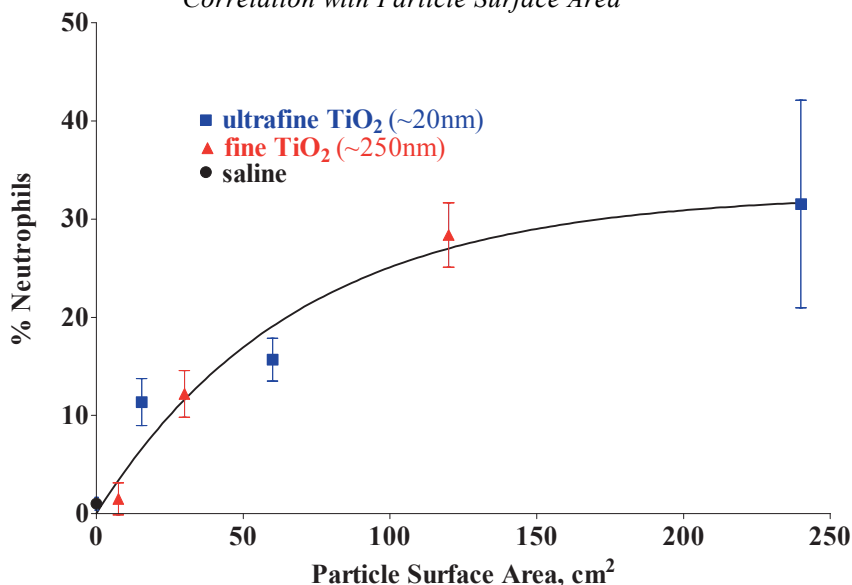*Correlation with Particle Surface Area*

Figure 3b. The same dose–response relationship but with dose expressed as particle surface area, indicating that particle surface area seems to be a more appropriate dosemetric for comparing effects of different sized particles provided they are of the same chemical structure (anatase TiO$_2$ in this case).

particle number (Oberdörster, 2000). Moreover, normalizing the particle surface dose to lung weight shows excellent agreement of the inflammatory response in both rats and mice (Fig. 3c). The better fit of dose–response relationships by expressing the dose as surface area rather than mass when describing toxicologic effects of inhaled solid particles of different sizes has been pointed out repeatedly, especially when particles of different size ranges – nano to fine – were studied (Brown et al. 2001; Donaldson et al. 1998, 2002; Driscoll 1996; Oberdörster and Yu 1990; Oberdörster et al. 1992a; Tran et al. 1998, 2000) However, it needs to be considered that other particle parameters, such as shape, surface chemistry, surface charge, agglomeration state, etc., influence effects aswell. In the case of TiO$_2$, photocatalytic activity also plays a role, as pointed out recently (Sayes et al. 2006). Thus, particle chemistry, and specifically surface chemistry plays a decisive role in addition to particle size.
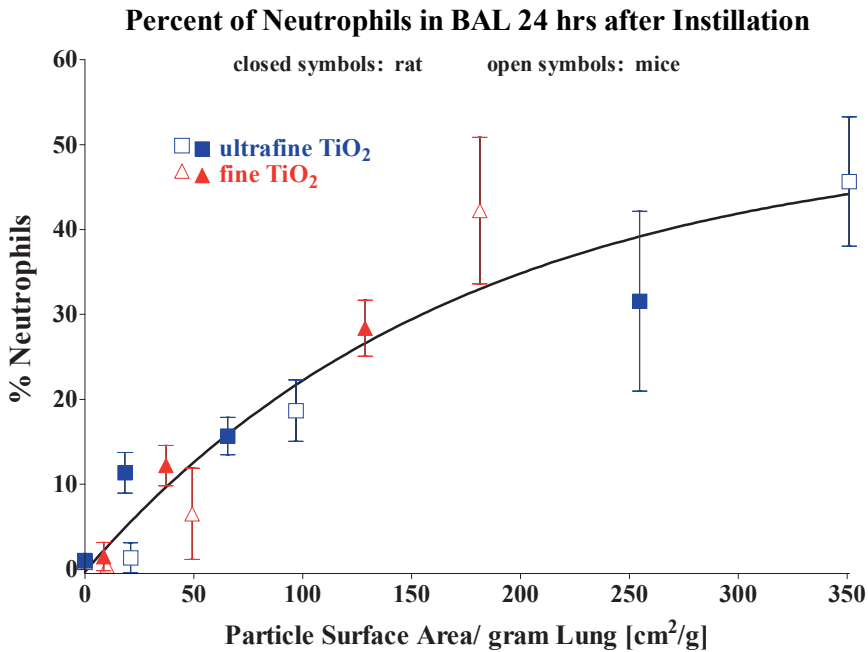
Percent of Neutrophils in BAL 24 hrs after Instillation

Figure 3c. Dose–response relationship of TiO$_2$ in rats and mice, normalized by particle surface area and lung weight. (20 nm TiO$_2$; ■ 250 nm TiO$_2$, ▲ saline control ●)

Engineered nanomaterials can have very different shapes, for example, spheres, fibers, tubes, rings, and planes. Toxicologic studies of spherical and fibrous particles have well established that natural (e.g., asbestos) and manmade (e.g., biopersistent vitreous) fibers are associated with increased risks of pulmonary fibrosis and cancer after prolonged exposures (Greim et al. 2001). Critical parameters are the three Ds: dose, dimension, and durability of the fibers. Fibers are defined as elongated structures with a diameter-tolength ratio (aspect ratio) of 1:3 or greater and with a length of >5 μm and diameter ≤3 μm (World Health Organization [WHO] 1985). Carbon nanotubes have aspect ratios of up to ≥100, and length can exceed 5 μm with diameters ranging from 0.7 to 1.5 nm for singlewalled nanotubes, and 2–50 nm for multiwalled nanotubes. Results from three studies using intratracheal dosing of carbon nanotubes in rodents indicate significant acute inflammatory pulmonary effects that either subsided in rats (Warheit et al. 2004) or were more persistent in mice (Lam et al. 2004; Shvedova et al. 2005). Administered doses were very high, ranging from 1 to 5 mg/kg in rats; in mice doses ranged from 3.3 to 16.6 mg/kg (Lam et al. 2004) or somewhat lower, from 0.3 to 1.3 mg/kg (Shvedova et al.

2004). Granuloma formation as a normal foreign body response of the lung to high doses of a persistent particulate material was a consistent finding in these studies. Metal impurities (e.g., iron) from the nanotube generation process may also have contributed to the observed effects. Although these *in vivo* first studies revealed high acute effects, including mortality, this was explained by the large doses of the instilled highly aggregated nanotubes that caused death by obstructing the airways and should not be considered a nanotubes effect per se (Warheit et al. 2004).

Future studies should be designed to investigate both effects and also the fate of nanotubes after deposition in the respiratory tract, preferentially by inhalation using well dispersed airborne nanotubes reflecting conditions at the workplace. In order to design the studies using appropriate dosing, it is necessary to assess the likelihood and degree of human exposure. It is of utmost importance to characterize human exposures in terms of the physicochemical nature, the aggregation state, and concentration (number, mass, surface area) of engineered nanomaterials and perform animal and *in vitro* studies accordingly. If using direct instillation into the lower respiratory tract, a large range of doses, which include expected realistic exposures of appropriately prepared samples, needs to be considered.

1.1.2. Portals of Entry and Target Tissues

Most of the toxicity research on NPs *in vivo* has been carried out in mammalian systems, with a focus on respiratory system exposures for testing the hypothesis that airborne UFPs cause significant health effects. With respect to NPs, other exposure routes, such as skin and GI tract, also need to be considered as potential portals of entry. Portal-of-entry–specific defense mechanisms protect the mammalian organism from harmful materials. However, these defenses may not always be as effective for NPs, as is discussed below.

2.   Respiratory Tract

In order to appreciate what dose the organism receives when airborne particles are inhaled, information about their deposition as well as their subsequent fate is needed. Here we focus on the fate of inhaled nanosized materials both within the respiratory tract itself and translocated out of the respiratory tract. There are significant differences between NPs and larger particles regarding their behavior during deposition and clearance in the respiratory tract.

2.1. EFFICIENT DEPOSITION OF INHALED NPs

The main mechanism for deposition of inhaled NPs in the respiratory tract is diffusion due to displacement when they collide with air molecules. Other deposition mechanisms of importance for larger particles, such as inertial impaction, gravitational settling, and interception, do not contribute to NP deposition, and electrostatic precipitation occurs only in cases where NPs carry significant electric charges. Figure 4 shows the fractional deposition of inhaled particles in the nasopharyngeal, tracheobronchial, and alveolar regions of the human respiratory tract under conditions of nose breathing during rest, based on a predictive mathematical model (International Commission on Radiological Protection [ICRP] 1994). These predictions apply to particles that are inhaled as singlet particles of a given size and not as aggregates; the latter obviously will have larger particle size and different deposition site. In each of the three
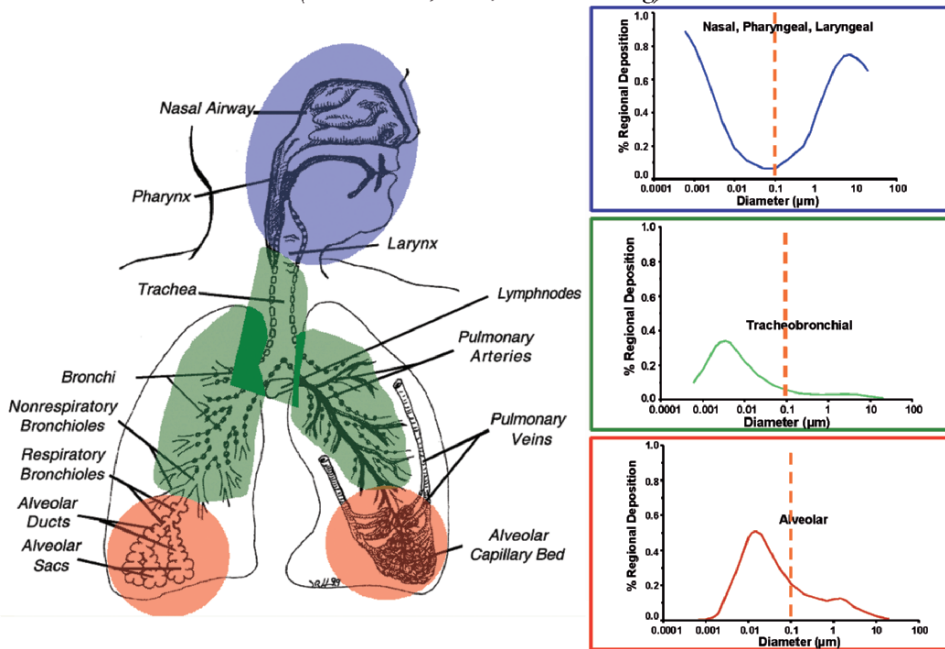

Figure 4. Predicted fractional deposition of inhaled particles in the nasopharyngeal, tracheo-bronchial, and alveolar region of the human respiratory tract during nose-breathing (based on data from ICRP 1994. (Drawing courtesy of J. Harkema.)

regions of the respiratory tract, significant amounts of a certain size of NPs (1–100 nm) are deposited. For example, 90% of inhaled 1 nm particles are deposited in the nasopharyngeal compartment, only approximately 10% in the tracheobronchial region, and essentially none in the alveolar region. On the other hand, 5 nm particles show about equal deposition of approximately 30% of the inhaled particles in all three regions; 20 nm particles have the highest deposition efficiency in the alveolar region (~50%), whereas in tracheobronchial and nasopharyngeal regions this particle size deposits with approximately 15% efficiency. These different deposition efficiencies should have consequences for potential effects induced by inhaled NPs of different sizes as well as for their disposition to extrapulmonary organs, as discussed further below.

2.1.1. *Disposition of NPs in the Respiratory Tract*

The preceding section summarized data demonstrating that inhaled NPs of different sizes can target all three regions of the respiratory tract. Several defense mechanisms exist throughout the respiratory tract aimed at keeping the mucosal surfaces free from cell debris and particles deposited by inhalation. Several reviews describe the well-known classic clearance mechanisms and pathways for deposited particles (Kreyling and Scheuch 2000; Schlesinger et al. 1997; U.S. EPA 2004), so here we only briefly mention those mechanisms and point out specific differences that exist with respect to inhaled NPs.

Once deposited, NPs – in contrast to larger-sized particles – appear to translocate readily to extrapulmonary sites and reach other target organs by different transfer routes and mechanisms. One involves transcytosis across epithelia of the respiratory tract into the interstitium and access to the blood circulation directly or *via* lymphatics, resulting in distribution throughout the body. The other is a not generally recognized mechanism that appears to be distinct for NPs and that involves their uptake by sensory nerve endings embedded in airway epithelia, followed by axonal translocation to ganglionic and CNS structures.

The most prevalent mechanism for clearance of solid fine and larger particles in the alveolar region is mediated by alveolar macrophages, through phagocytosis of deposited particles. In contrast, deposited particles of less than 100 nm are not efficiently cleared by this mechanism (Fig. 5). The success of macrophage–particle encounter appears to be facilitated by chemotactic attraction of alveolar macrophages to the site of particle deposition (Warheit et al. 1988). The chemotactic signal is most likely complement protein 5a (C5a), derived from activation of the complement cascade from serum proteins present on the alveolar surface (Warheit et al. 1986; Warheit and Hartsky 1993). This is followed by gradual movement of the macrophages with internalized particles toward the mucociliary escalator. The retention half-time of solid particles in
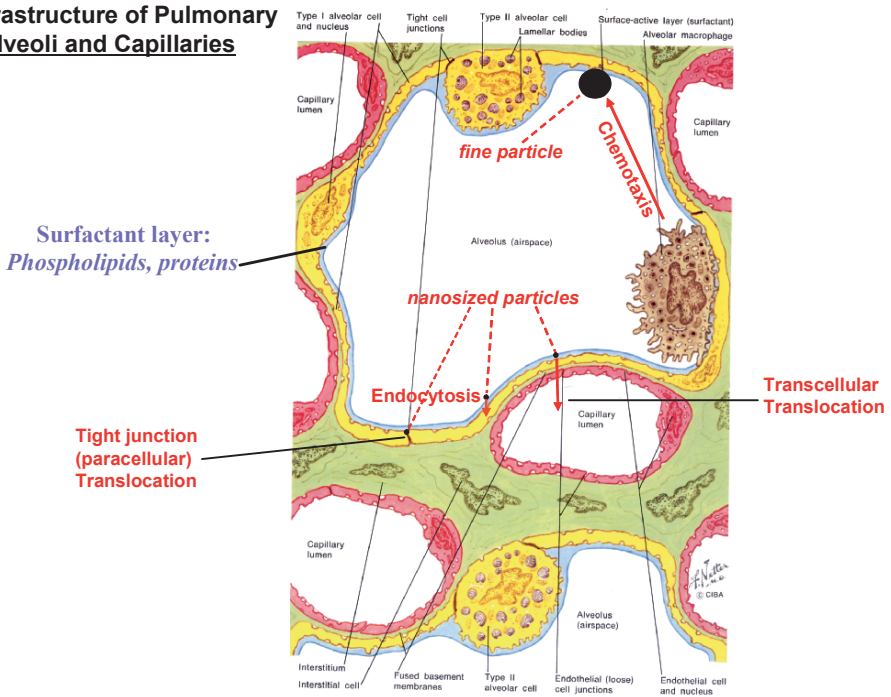
Figure 5. Phagocytosis and clearance of particles by alveolar macrophages is effective for fine particle, but inefficient for nanoparticles (<100 nm).

the alveolar region based on this clearance mechanism is about 70 days in rats and up to 700 days in humans. The efficacy of this clearance mechanism depends highly on the efficiency of alveolar macrophages to "sense" deposited particles, move to the site of their deposition, and then phagocytize them. This process of phagocytosis of deposited particles takes place within a few hours, so by 6–12 h after deposition essentially all of the particles are phagocytized by alveolar macrophages, to be cleared subsequently by the slow alveolar clearance mentioned above. However, it appears that there are significant particle size–dependent differences in the cascade of events leading to effective alveolar macrophage-mediated clearance. Figure 6 displays results of several studies in which rats were exposed to different-sized particles (for the 3 and 10 µm particles, 10 µg and 40 µg polystyrene beads, respectively, were instilled intratracheally) (Kreyling et al. 2002; Oberdörster et al. 1992b, 2000; Semmler et al. 2004). After 24 h, the lungs of the animals were lavaged repeatedly, retrieving about 80% of the total macrophages as determined in earlier lavage
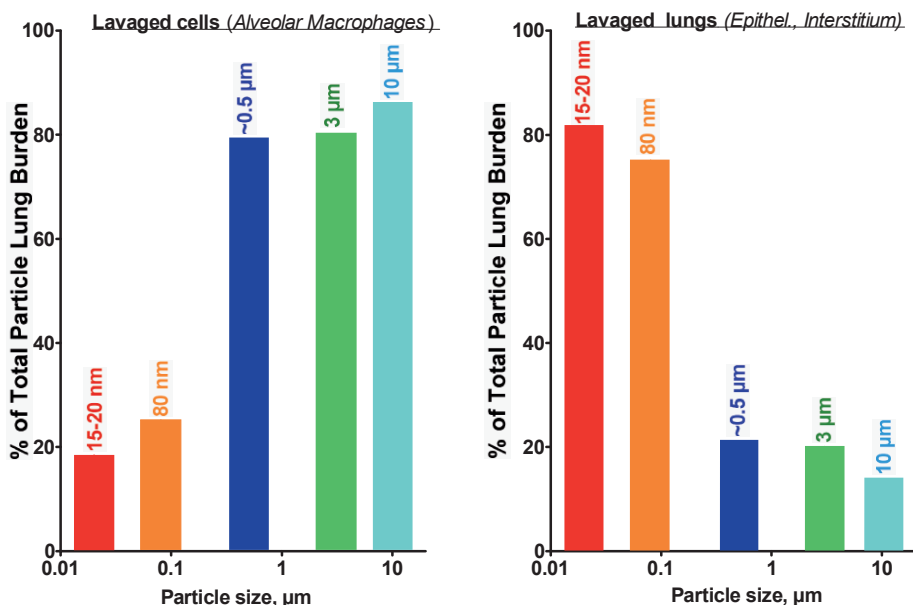
Figure 6. In vivo retention of inhaled nanosized and larger particles in alveolar macrophages (left side) and in exhaustively lavaged lungs (epithelial and interstitial retention, right side) 24 h post exposure. The alveolar macrophage is a most important defense mechanism in the alveolar region for fine and coarse particles, yet inhaled singlet NP are not efficiently phagocytized by alveolar macrophages.

experiments (Ferin et al. 1991). As shown in Fig. 6, approximately 80% of 0.5, 3, and 10 μm particles could be retrieved with the macrophages, whereas only approximately 20% of nanosized 15–20 nm and 80 nm particles could be lavaged with the macrophages. In effect, approximately 80% of the UFPs were retained in the lavaged lung after exhaustive lavage, whereas approximately 20% of the larger particles >0.5 μm remained in the lavaged lung. This indicates that NPs either were in epithelial cells or had further translocated to the interstitium.

2.1.2. *Epithelial Translocation*

Because of the apparent inefficiency of alveolar macrophage phagocytosis of NPs, one might expect that these particles interact instead with epithelial cells. Indeed, results from several studies show that NPs deposited in the respiratory tract readily gain access to epithelial and interstitial sites. This was also shown in studies with ultrafine PTFE fumes: shortly after a 15 min exposure, the

fluorine-containing particles could be found in interstitial and submucosal sites of the conducting airways as well as in the interstitium of the lung periphery close to the pleura (Oberdörster 2000). Such interstitial translocation represents a shift in target site away from the alveolar space to the interstitium and to the lymph and blood circulation, potentially causing direct particle induced effects there.

2.1.3. Translocation to the Circulatory System

Once the particles have reached pulmonary interstitial sites, uptake into the blood circulation, in addition to lymphatic pathways, can occur; again, this pathway is dependent on particle size, favoring NPs. Berry et al. (1977) were the first to describe translocation of NPs across the alveolar epithelium using intratracheal instillations of 30 nm gold particles in rats. Within 30 min postexposure, they found large amounts of these particles in platelets of pulmonary capillaries; the researchers suggested that this is an elimination pathway for inhaled particles that is significant for transporting the smallest air-pollutant particles – in particular, particles of tobacco smoke – to distant organs. They also hypothesized that this "might predispose to platelet aggregation with formation of microthrombi atheromatous plaques" (Berry et al. 1977). Since then, a number of studies with different particle types have confirmed the existence of this translocation pathway, as summarized in Table 4.

Collectively, these studies indicate that particle size and surface chemistry (coating), and possibly charge, govern translocation across epithelial and endo-thelial cell layers. In particular, the studies summarized by Mehta et al. (2004) and those performed by Heckel et al. (2004) using intravenous administration of albumin-coated gold NPs in rodents demonstrated receptor-mediated transcytosis (albumin-binding proteins) via caveolae. These 50–100 nm vesicles, first described by Simionescu et al. (1975), form from indentations of the plasmalemma and are coated with the caveolin-1 protein. Albumin, as the most abundant protein in plasma and interstitium, appears to facilitate NP endocytosis, as does lecithin, a phospholipid: even 240 nm polystyrene particles translocated across the alveolo-capillary barrier when coated with lecithin, whereas uncoated particles did not (Kato et al. 2003). The presence of both albumin and phospholipids in alveolar epithelial lining fluid may, therefore, be important constituents for facilitated epithelial cell uptake of NPs after deposition in the alveolar space.

However, as shown by results summarized in Table 4, surface coating of NPs with albumin clearly causes even the smallest particles to be internalized via caveolae. The presence of caveolae on cells differs: they are abundant in lung capillaries and alveolar type l cells but not in brain capillaries (Gumbleton 2001). In the lung, during inspiratory expansion and expiratory contraction of the alveolar walls, caveolae with openings around 40 nm disappear and reappear, forming

TABLE 4. Particle Size and Surface Chemistry-related Alveolar-Capillary Translocation.

Particle size (nm)	Type	Translocation	Localization/effect	Reference
5–20	Gold, albumin coated	Yes	Via caveolae	Mehta et al. (2004)
8	Gold, albumin coated	Yes	Via "vesicles"	König et al. (1993)
8	Gold, albumin coated	Yes	Via caveolae	Heckel et al. (2002)
18	Iridium	Yes[A]	Extrapulmonary organs	Kreyling et al. (2002)
30	Gold	Yes	Platelet	Berry et al. (1977)
35	Carbon	Yes	Liver	Oberdörster et al. (2002)
60	Polystyrene, positive charge	Yes[B]	Thrombus, early	Nemmar et al. (2002) Silva et al. (2005)
60	Polystyrene, negative charge	?	No thrombus	Nemmar et al. (2002)
80	Iridium	Yes[A]	Extrapulmonary organs	Kreyling et al. (2002)
240	Polystyrene, lecithin	Yes	Monocyte	Kato et al. (2003)
240	Polystyrene, uncoated	No		Kato et al. (2003)
400	Polystyrene	No	No thrombus	Nemmar et al. (2004)
	Surface coating (chemistry) charge, size govern translocation			

[A]minimal; [B]indirect evidence

vesicles that are thought to function as transport pathways across the cells for macromolecules (Patton 1996). Knowledge from virology about cell entry of biologic NSPs (viruses) via clathrin-coated pits and caveolae mechanisms should also be considered (Smith and Helenius 2004) and can shed light on the mechanism by which engineered NPs may enter cells and interact with sub-cellular structures.

Evidence in humans for the translocation of inhaled NPs into the blood circulation is ambiguous, with one study showing rapid appearance in the blood and significant accumulation of label in the liver of humans inhaling ^{99}Tc-labeled 20 nm carbon particles (Nemmar et al. 2002), whereas other studies using the same labeled particles reported no such accumulation (Brown et al. 2002; Mills et al. 2006). Taking into consideration all of the evidence from animal and human studies for alveolar translocation of NPs, it is likely that this pathway also exists in humans; however, the extent of extrapulmonary translocation is highly dependent on particle surface characteristics/chemistry, in addition to particle size. Translocation to the blood circulation could provide a mechanism for a

direct particle effect on the cardiovascular system as an explanation for epidemiologic findings of cardiovascular effects associated with inhaled ambient UFPs (Pekkanen et al. 2002; Wichmann et al. 2000) and for results of clinical studies showing vascular responses to inhaled elemental carbon UFPs (Pietropaoli et al. 2004). In addition to direct alveolar translocation of NSPs, cardiovascular effects may also be the corollary of a sequence of events starting with particle-induced alveolar inflammation initiating a systemic acute phase response with changes in blood coagulability and resulting in cardiovascular effects (Seaton et al. 1995) (Fig. 7).
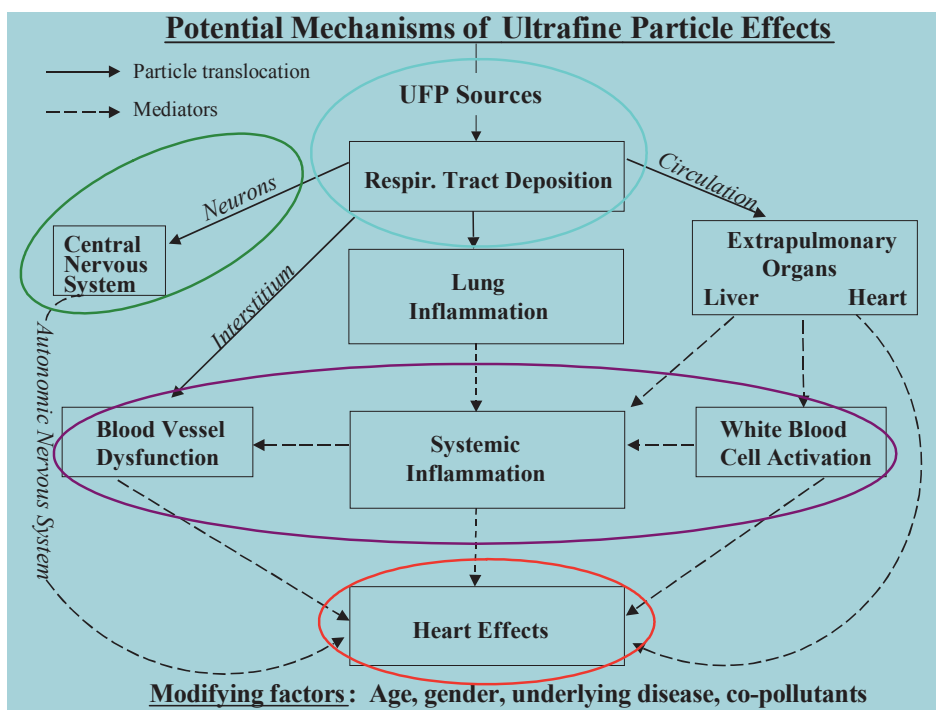

Figure 7. Potential mechanisms of ultrafine particle effects.

Once NSPs have translocated to the blood circulation, they can be distributed throughout the body. The liver is the major distribution site via uptake by Kupffer cells, followed by the spleen as another organ of the reticuloendothelial system, although coating with polyethylene glycol (PEG) almost completely prevents hepatic and splenic localization so that other organs can be targeted (Akerman et al. 2002). Distribution to heart, kidney, and immune-modulating organs (spleen, bone marrow) has been reported. For example, several types of NPs, ranging from 10 to 240 nm, localized to a significant degree in bone marrow after intravenous injection into mice (Table 5).

TABLE 5. Translocation of Nano-Sized Particles in the Blood Circulation to Bone Marrow In Mice.

Particle size (nm)	Type	Finding	Reference
~10	PEG-quantum dots	Fast appearance of QDs in liver, spleen, lymph nodes, and bone marrow (mouse)	Ballou et al. (2004)
<220	Metallo-fullerene	Highest accumulation in bone marrow after liver; continued increase in bone marrow but decreases in liver (mouse)	Cagle et al. (1999)
90–250	HAS-coated polylactic acid nanoparticles	Significant accumulation in bone marrow, second to liver (rat)	Bazile et al. (1992)
240	Polystyrene (non-biodegradable) polylisohexylcyonacrylate (biodegradable)	Rapid passage through endothelium in bone marrow, uptake by phagocytizing cells in tissue (mouse)	Gibaud et al. (1996, 1998, 1994)

Such target specificity may be extremely valuable for drug delivery; for example, drug delivery to the CNS via blood-borne NPs requires NP surface modifications in order to facilitate translocation across the tight blood–brain barrier via specific receptors (e.g., apolipoprotein coating for LDL-receptor–mediated endocytosis in brain capillaries) (Kreuter 2001, 2004; Kreuter et al. 2002). Such highly desirable properties of NPs must be carefully weighed against potential adverse cellular responses of targeted NP drug delivery, and a rigorous toxicologic assessment is mandatory.

2.1.4. Neuronal Uptake and Translocation

A translocation pathway via neuronal structures for solid particles in the respiratory tract involving neuronal axons is apparently specific for NPs. Respective studies are summarized in Table 6.

TABLE 6. Studies of Neuronal Translocation of Nano-sized Particles from Respiratory Tract.

1941	*Bodian and Howe*: *Olfactory* axonal transport of Polio-virus (30 nm) after intranasal instillation in chimpanzee. Transport velocity: 2.4 mm/h
1970	*de Lorenzo*: *Olfactory* axonal transport of 50 nm colloidal gold after intranasal instillation in squirrel monkey. Transport velocity: 2.5 mm/h
1998	*Hunter and Undem*: Rhodamine-labeled 40 nm microspheres translocation via sensory nerves of *TB region* to ganglion nodosum in hamster after intratracheal instillation.
1999	*Hunter and Dey*: Retrograde tracing of trigeminal neurons from nasal epithelium with Rhodamine-labelled ~40 nm microspheres after intratracheal instillation.
2004	*Oberdörster et al.*: 13C particles (CMD ~36 nm) translocation to *olfactory* bulb after inhalation exposure in rats.
2006	*Elder et al.*: Mn-oxide particles (CMD ~30 nm) inhalation exposure in rats; increased Mn and inflammatory response in olfactory bulb.

This pathway was described more than 60 years ago, yet it has received little or no attention from toxicologists. This pathway comprises sensory nerve endings of the olfactory and the trigeminus nerves and an intricate network of sensory nerve endings in the tracheobronchial region. These early studies concerned a large series of studies with 30-nm polio virus intranasally instilled into chimpanzees and rhesus monkeys (Bodian and Howe 1941a, b; Howe and Bodian 1940). Their studies revealed that the olfactory nerve and olfactory bulbs are, indeed, portals of entry to the CNS for intranasally instilled nanosized polio virus particles, which could subsequently be recovered from the olfactory bulbs. The close proximity of nasal olfactory mucosa and olfactory bulb requires only a short distance to be covered by neuronal transport (Fig. 8). Bodian and Howe (1941b) determined the transport velocity of the virus in the axoplasm of axons to be 2.4 mm/h, which is very well in agreement with neuronal transport velocities measured later by Adams and Bray (1983) for solid particles (up to 500 nm) directly microinjected into giant axons of crabs, and by de Lorenzo (1970) for silver-coated colloidal gold (50 nm) in squirrel monkeys.
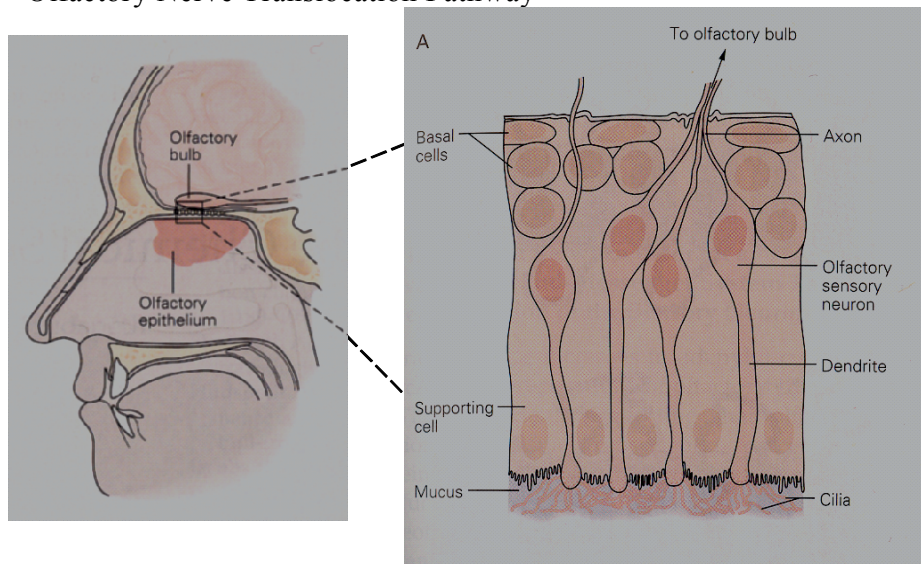
Olfactory Nerve Translocation Pathway

Figure 8. Close proximity of olfactory mucosa to olfactory bulb of the CNS. Inhaled NP, especially below 10 nm, deposit efficiently on the olfactory mucosa by diffusion, similar to airborne "smell" molecules which deposit in this area of olfactory dendritic cilia. Subsequent uptake and translocation of solid NP along axons of the olfactory nerve has been demonstrated in nonhuman primates and rodents. Surface chemistry of the particles may influence their neuronal translocation. (From Kandel et al. 2000.)

The de Lorenzo (1970) study demonstrated in squirrel monkeys that intranasally instilled silver-coated colloidal gold particles (50 nm) translocated anterogradely in the axons of the olfactory nerves to the olfactory bulbs. The 50 nm gold particles even crossed synapses in the olfactory glomerulus to reach mitral cell dendrites within 1 h after intranasal instillation. An interesting finding in this study – and important for potential adverse effects – was that the NPs in the olfactory bulb were no longer freely distributed in the cytoplasm but were preferentially located in mitochondria.
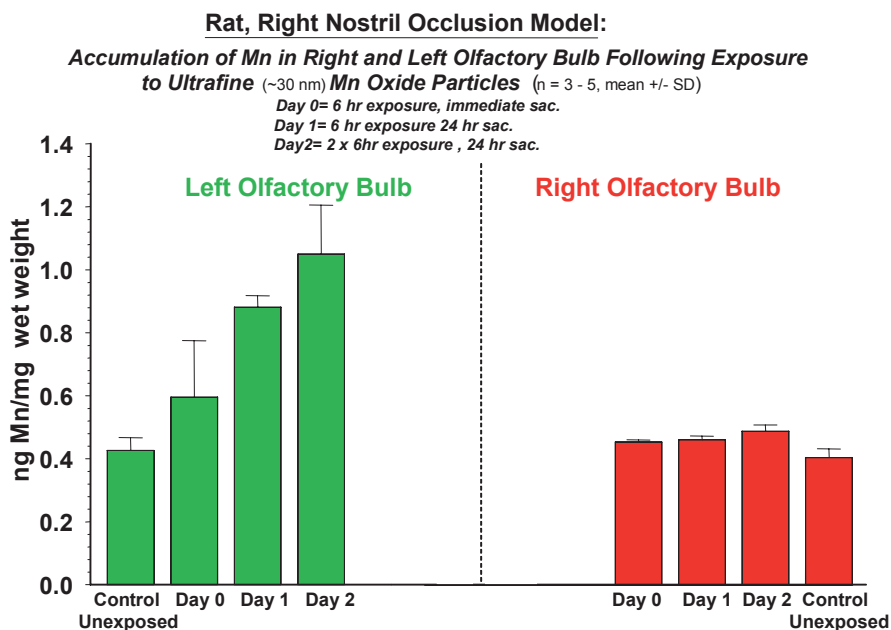

Rat, Right Nostril Occlusion Model:

Accumulation of Mn in Right and Left Olfactory Bulb Following Exposure to Ultrafine (~30 nm) *Mn Oxide Particles* (n = 3 - 5, mean +/- SD)

Figure 9. Occlusion of the right nostril of rats during 6 h inhalation of nanosized Mn-oxide particles (~30 nm CMD, ~450 μg/m^3) resulted in accumulation of Mn only in the left olfactory bulb only at 24 h after dosing. (From Elder et al. 2006.)

Newer studies indicated that this translocation pathway is also operational for inhaled NPs. Inhalation of elemental ^{13}C UFPs (CMD = 35 nm) resulted in a significant increase of ^{13}C in the olfactory bulb on day 1, which increased further throughout day 7 postexposure (Oberdörster et al. 2004). Results of another inhalation study with solid nanosized (CMD = 30 nm) manganese oxide particles in rats showed after a 12 day exposure to ~450 μg/m^3 (6 h/day, 5 days/week) a more than 3.5-fold significant increase of Mn in the olfactory bulb, compared with only a doubling of Mn in the lung. When one nostril was occluded during a 6-h exposure, Mn accumulation in the olfactory bulb was

restricted to the side of the open nostril only (Fig. 9) (Elder et al. 2006). This result contrasts with 15 day inhalation of larger-sized MnO_2 particles in rats (1.3 and 18 μm mass median aerodynamic diameter) where no significant increases in olfactory Mn was found (Fechter et al. 2002). This was to be expected given that the individual axons of the fila olfactoria (forming the olfactory nerve) are only 100–200 nm in diameter (de Lorenzo 1957; Plattig 1989). The most recent study by Elder et al. (2006) with inhaled Mn-oxide also showed significant increases of TNF-α message and protein in the olfactory bulb (Fig. 10).

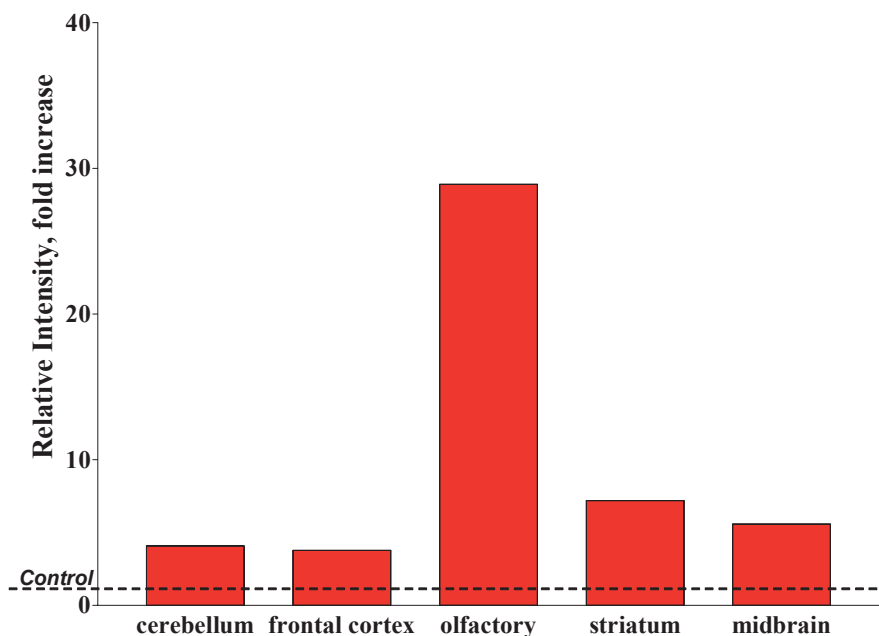*Figure 10*. Brain region TNF-α protein expression changes after 12 days of exposure to ultrafine Mn-oxide in rats. (From Elder et al. 2006.)

Translocation into deeper brain structures may possibly occur as well, as shown in rats for soluble Mn (Gianutsos et al. 1997), was not observed in a recent inhalation study with soluble Mn in monkeys (Dorman et al. 2006). Further evidence for movement of NPs along axons and dendrites in humans is provided by knowledge accumulated by virologists who have long understood the movement of human meningitis virus through olfactory and trigeminal neurons and, the trigeminal neuron to trigger outbreaks of herpes cold sores in humans (Kennedy and Chaudhuri 2002; Terasaki et al. 1997).

There are additional neuronal translocation pathways for solid NPs via the trigeminus nerve and tracheobronchial sensory nerves (Table 6). A study by Hunter and Dey (1998) in rats demonstrated the translocation of intranasally instilled rhodamine-labeled microspheres (20–200 nm) to the trigeminal ganglion inside the cranium via uptake into the ophthalmic and maxillary branches of the trigeminus nerve that supplies sensory nerve endings throughout the nasal mucosa. In another study, Hunter and Undem (1999) instilled the same microparticles intratracheally into guinea pigs; they found neuronal translocation of these solid microparticles to the ganglion nodosum in the neck area that is networked into the vagal system. This finding may be relevant for ambient UFPs because it can be hypothesized that cardiovascular effects associated with ambient particles in epidemiologic studies (Utell et al. 2002) are in part due to direct effects of translocated UFPs on the autonomic nervous system via sensory nerves in the respiratory tract.

In the context of potential CNS effects of air pollution, including ambient UFPs, two recent studies with exposures of mice to concentrated ambient fine particles and UFPs should be mentioned. Campbell et al. (2005) and Veronesi et al. (2005) found significant increases of tumor necrosis factor-α or decreases in dopaminergic neurons, supporting the hypothesis of ambient PM causing neurodegenerative disease. A study by Calderon-Garcidueñas et al. (2002) may also point to an interesting link between air pollution and CNS effects: these authors described significant inflammatory or neurodegenerative changes in the olfactory mucosa, olfactory bulb, and cortical and subcortical brain structures in dogs from a heavily polluted area in Mexico City, whereas these changes were not seen in dogs from a less-polluted rural control city. However, whether direct effects of airborne UFPs are the cause of these effects remains to be determined.

Although the existence of neuronal translocation of NPs has been well established, size alone is only one particle parameter governing this process. Surface characteristics of NPs (chemistry, charge, shape, aggregation) are essential determinants as well, and it should not be assumed that all NPs, when inhaled, will be distributed by the mechanism described here. It should be kept in mind, however, that the unique biokinetic behavior of NPs – endocytosis, transcytosis, neuronal and circulatory translocation and distribution – which makes them desirable for medical therapeutic or diagnostic applications – may be associated with potential toxicity. For example, NP-facilitated drug delivery to the CNS raises the question of the fate of NPs after their translocation to specific cell types or to subcellular structures in the brain. For example, does mitochondrial localization induce oxidative stress? How persistent is the coating or the core of the NPs? A respective safety evaluation is key.

3.   Exposure via Skin

A potentially important uptake route is through dermal exposure (Table 7).

TABLE 7. Skin as a Portal-of-Entry for Nanoparticles.

Translocation pathways to living tissue
• *across cells of stratum corneum*
• *between cells of stratum corneum*
• *via hairshaft follicle*
• *via sweat glands*
• *through inflamed /injured skin*

Distribution from epidermal/dermal region
• *lymphatic vessels*
• *blood vessels*
  –venous
  –arterial
  –sensory neurons
  –touch
  –pain
  –pressure
  –warmth

The epidermis, consisting of the outer horny layer (stratum corneum), the prickle cell layer (stratum spinosum), and basal cell layer (stratum basale), forms a very tight protective layer for the underlying dermis (Fig. 11). The dermis has a rich supply of blood and tissue macrophages, lymph vessels, dendritic cells (Langerhans, also in stratum spinosum of epidermis), and five different types of sensory nerve endings. Broken skin represents a readily available portal of entry even for larger (0.5–7 μm) particles, as evidenced by reports about accumulation of large amounts of soil particles in inguinal lymph nodes of people who often run or walk barefoot; this can be associated with elephantiatic lymphedema (podoconiosis) (Corachan et al. 1988; Blundell et al. 1989). Tinkle et al. (2003) hypothesized that unbroken skin when flexed–as in wrist movements–would make the epidermis permeable for NPs. They demonstrated in a proof-of-concept experiment that, indeed, flexing the skin, but not flat skin, resulted in penetration of even 1 μm fluorescent beads to the dermis. The follow-up question about access of particles in the dermis to the circulation is answered by the aforementioned reports of podoconiosis, that is, uptake into the

lymphatic system and regional lymph nodes. Subsequent translocation of NPs beyond lymph nodes to the blood circulation is likely to occur as well, as shown in studies with small asbestos fibers (Oberdörster et al. 1988).
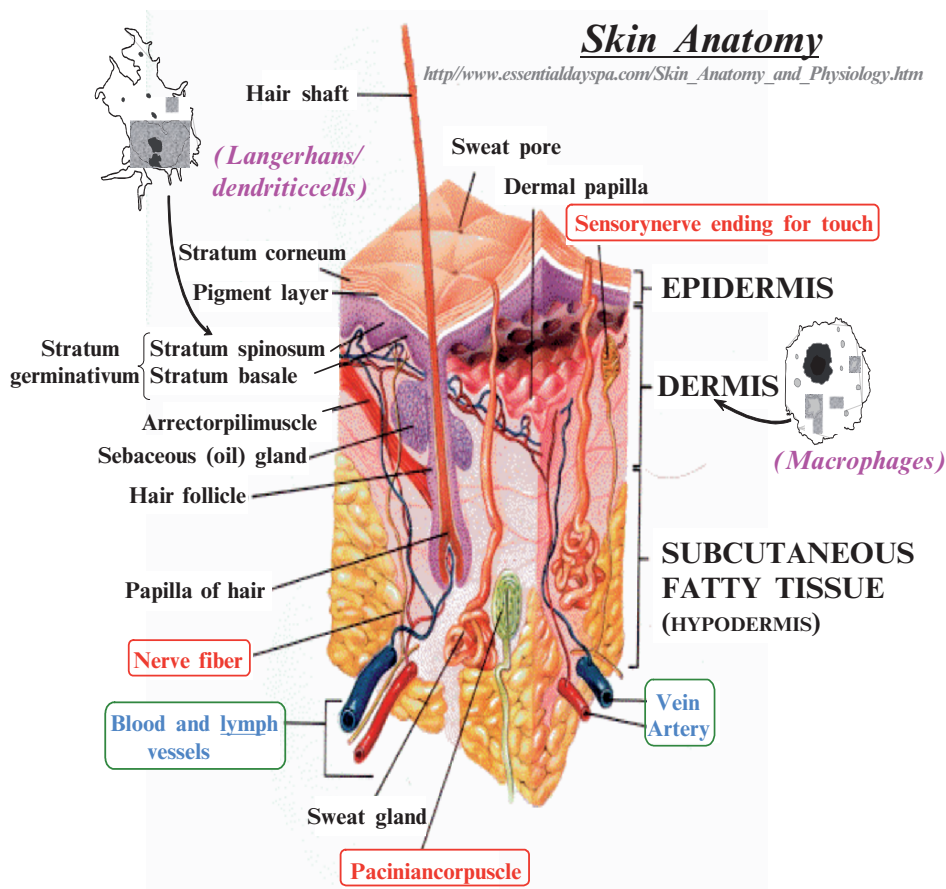

Figure 11. The epidermis represents a tight barrier against NSP penetration. Quantitatively, dermal translocation will, therefore, be minimal or nonexistent under normal conditions but increases in areas of skin flexing (Tinkle et al. 2003) and broken skin. Once in the dermis, lymphatic uptake is a major translocation route, likely facilitated by uptake in dendritic cells (epidermis) and macrophages; other potential pathways may include the dense networks of blood circulation and sensory nerves in the dermis. (Figure adapted from: http/www.essential dayspa.com/Skin_Anatomy_and_ Physiology.htm).

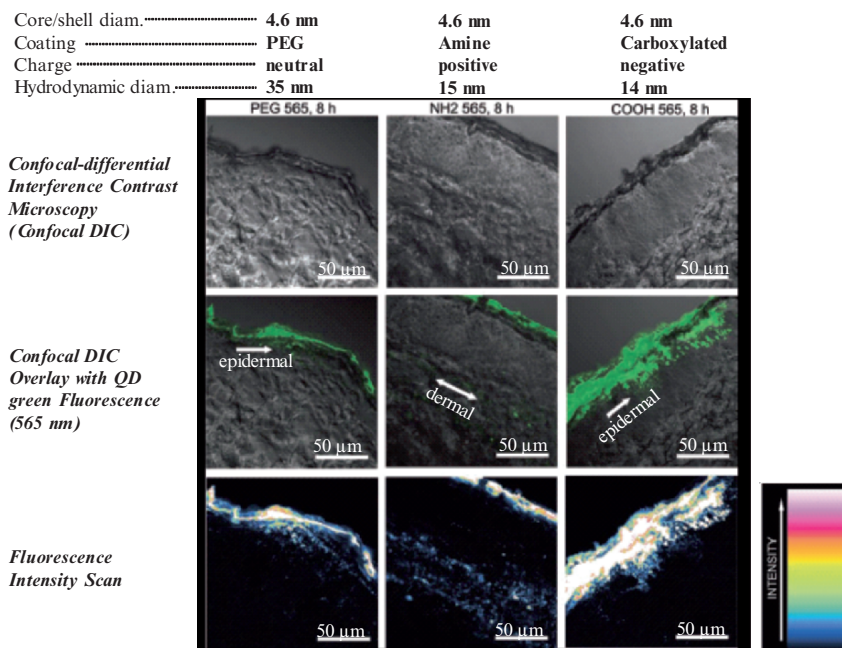| Core/shell diam.	4.6 nm	4.6 nm	4.6 nm
Coating	PEG	Amine	Carboxylated
Charge	neutral	positive	negative
Hydrodynamic diam.	35 nm	15 nm	14 nm

Figure 12. Quantum dots (*Invitrogen*) penetration of pig skin after 8 h treatment. (From Ryman-Rasmussen et al. 2006.)

A recent study by Ryman-Rasmussen et al. (2006) demonstrated translocation of quantum dots in a pig skin ex vivo model (Fig. 12): Quantum dots of neutral, positive and negative charge were found to penetrate to dermis and epidermis, however, the amount being translocated within 8 h could not be quantitated and is likely to be very low. Using confocal laser scanning microscopy, Verma et al. (2003) also showed that nano liposomes, depending on their size, did penetrate human abdominal skin.

Recent studies by Kim et al. (2004) in mice and pigs with intradermally injected near infrared quantum dots confirmed that NPs, once in the dermis, will localize to regional lymph nodes, which makes these particles very useful for *in vivo* imaging. Likely transport mechanisms to the lymph nodes are skin macrophages and dendritic (Langerhans) cells (Ohl et al. 2004; Sato et al. 1998); this raises a question about potential modulation of immune responses, after interaction of these NP-containing macrophages and dendritic cells with T lymphocytes. For example, Chen et al. (1998) were able to raise antibodies in mice specific for C60 after intraperitoneal injections of C60 conjugated to thyroglobulin and serum albumin. Clearly, research is needed to determine whether and under what conditions NPs can be recognized by the immune

system, following any route of uptake into the organism. Another question relates to the potential of sensory skin nerves to take up and translocate NPs. Given that this mechanism has been demonstrated for the nasal and tracheobronchial regions of the respiratory tract, how likely is this to occur in the dermis layer of the skin with its dense supply of different types of considering data on neuronal uptake and translocation of NPs after intra-muscular injection. For example, nanosized ferritin and iron-dextran, after injection into the tongue of mice, labeled the neurons of the hypoglossal nuclei, and injection of both of these NPs into facial muscles of mice also resulted in synaptic uptake; cationized ferritin was also detected in cell bodies of facial neurons, indicating that electrical charge is of importance for incorporation into axons and axonal transport (Arvidson 1994; Malmgren et al. 1978; Olsson and Kristensson 1981). Other studies using intramuscular injection of ferritin (~112 nm), irondextran (11 or 21 nm), and gold protein (20–25 nm) NPs also showed rapid penetration through the basal lamina into the synaptic clef of the neuromuscular junction, but this was restricted to only the smaller NPs, implying that there may be a size-dependent penetration of the basal lamina with a threshold somewhere between 10 and 20 nm (Oldfors and Fardeau 1983).
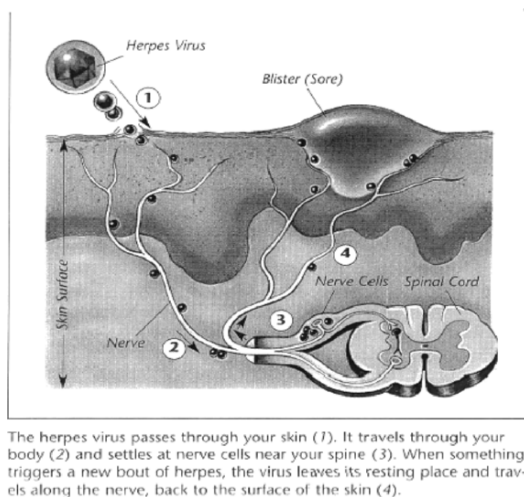

The herpes virus passes through your skin (1). It travels through your body (2) and settles at nerve cells near your spine (3). When something triggers a new bout of herpes, the virus leaves its resting place and trav-els along the nerve, back to the surface of the skin (4).

Figure 13. Dermal translocation of nanosized materials may follow the same pathways taken by viruses. (From: "Planning your Pregnancy and Birth" 3rd edn. American College of OB/GYN.)

Neuronal transport of NPs along sensory skin nerves is well established for herpes virus (Fig. 13). After passing through the skin – especially broken skin –

the viruses are transported retrogradely along dendrites of sensory neurons to the dorsal root ganglion, where they remain dormant until a stress situation triggers anterograde translocation along the dendrites back to the skin (Kennedy and Chaudhuri 2002; Terasaki et al. 1997). Future studies need to determine whether and to what degree such translocation along sensory skin neurons also occurs with NPs penetrating the epidermis.

4. Subcellular Distribution

NPs can enter cells via different endocytotic mechanisms, as outlined in previous sections, involving specific sites on the cell's surface such as caveolae or clathrin-coated pits. Newer data also demonstrated non-endocytotic diffusional cell uptake for nano-sized particles (Geiser et al. 2005). Once in the cytoplasm, there are several possibilities of interacting with subcellular structures, the cytoskeleton, the mitochondria, the endoplasmic reticulum, the golgi apparatus, and the nucleus itself. Localization of nano-sized materials in mitochondria after entry into cells has been shown by several investigators (Table 8; Fig. 14).
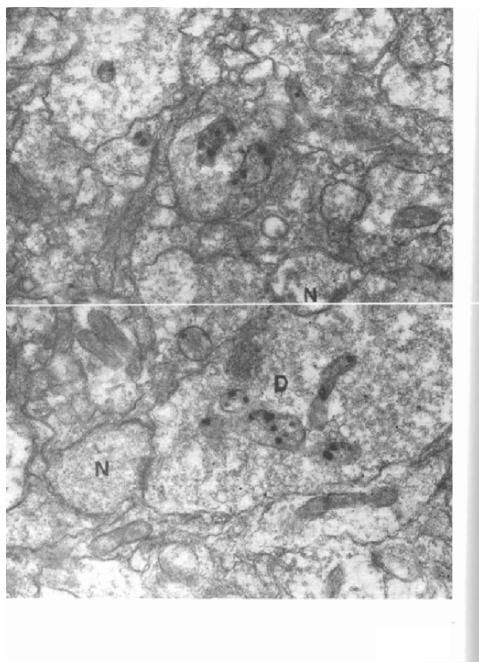

Figure 14. Squirrel monkey, intranasal colloidal gold particles (50 nm): Translocation to mitochondria of dendrites in mitral cells (D) of olfactory bulb after crossing the olfactory nerve (N)/mitral cell synapse. (From de Lorenzo, 1970.)

TABLE 8. Mitochondrial Localization after Dosing with Nano-sized Particles.

Material and Cell Type	Reference
Gold nanoparticles, squirrel monkey mitral cells of olfactory bulb	de Lorenzo (1970)
Colloidal gold, Rhesus monkey, sustentacular cells of olfactory mucosa	Gopinath et al. (1978)
Fullerene derivative, *in vitro*, fibroblast cell line	Foley et al. (2002)
Ambient UFP, *in vitro*, macrophage cell line	Li et al. (2003)
Micellar nanocontainers (*Block copolymer micelles*), *in vitro*, pheochromocytoma cells	Rodoslav et al. (2003)

4.1. WILL OXIDATIVE STRESS BE INDUCED?

Depending on particle chemistry, they may induce oxidative stress responses even resulting in apoptotic response, as shown by Li et al. (2003) in vitro using a macrophage cell line exposed to ambient UFP.
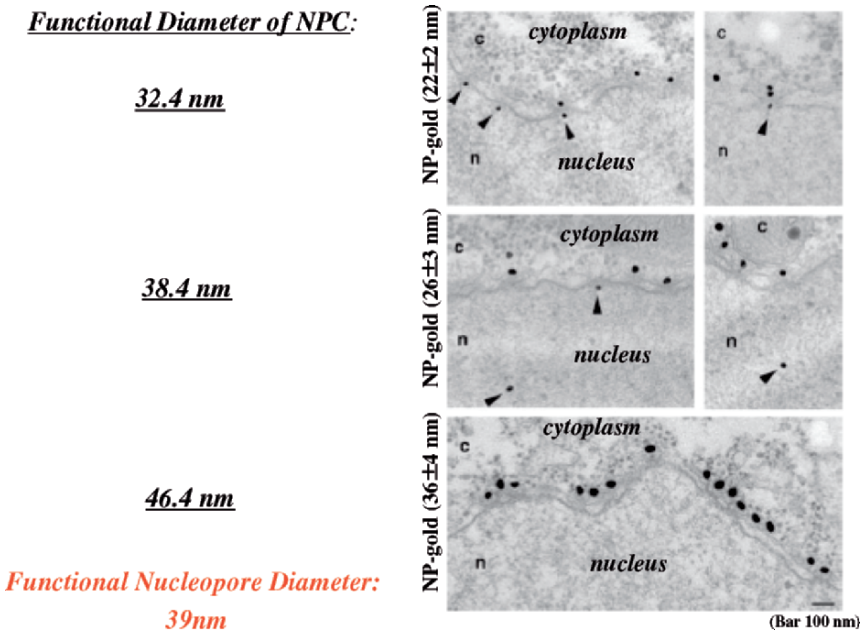

Figure 15. Nuclear import of nucleoplasmin-coated gold particles into oocyte nuclei via NPC. (From Panté and Kann, 2002.)

NP entry into the nucleus via the nucleopore complex (NPC) is another possibility. Using different sizes of nanogold particles, Panté and Kann (2002) determined the functional diameter of the NPC to be 39 nm (Fig. 15). Subsequently, Tsoli et al. (2005) reported that melanoma cells incubated with 1.4 nm gold particles (consisting of only 55 gold atoms) showed concentration dependent increased cytotoxicity, associated with nuclear disposition of these NPs. In fact, 50% of the gold particles in the nucleus was irreversibly attached to the major grooves of B-DNA (Fig. 16). The authors attribute the observed high cytotoxicity to the linking of the 1.4 nm particles to DNA. Confirmatory *in vivo* studies still need to be carried out; it would be of interest to determine as to whether the finding of gold–DNA interaction is specific for gold as the most electronegative metal linking with the negatively charged vicinity in the DNA groove (Tsoli et al. 2005)
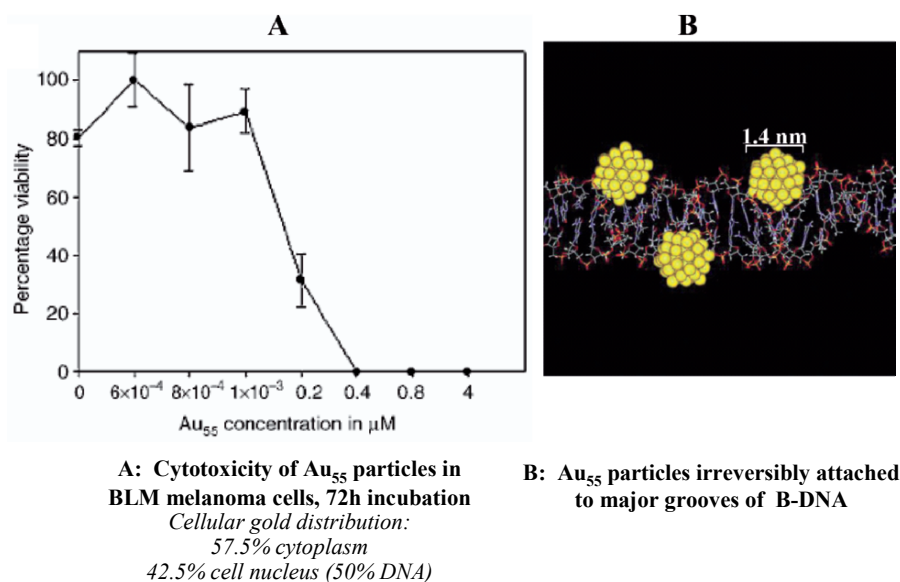

A: Cytotoxicity of Au_{55} particles in BLM melanoma cells, 72h incubation
Cellular gold distribution:
57.5% cytoplasm
42.5% cell nucleus (50% DNA)

B: Au_{55} particles irreversibly attached to major grooves of B-DNA

Figure 16. Cellular uptake and toxicity of Au_{55} nanoparticles (1.4 nm). (From Tsoli et al. 2005.)

5. Summary and Outlook

The biokinetics of NPs are different from larger particles. When inhaled, they are efficiently deposited in all regions of the respiratory tract; they evade

specific defense mechanisms; and they can translocate out of the respiratory tract via different pathways and mechanisms involving endocytosis and transcytosis, including uptake and transport via neuronal structures and distribution via the blood circulation. When in contact with skin, there is evidence of penetration to the dermis followed by translocation via lymph to regional lymph nodes. A possible uptake into sensory nerves needs to be investigated. When ingested, systemic uptake via lymph into the organism can occur, but most ingested NP are excreted via feces. When in blood circulation, they can distribute throughout the organism, and they are taken up into liver, spleen, bone marrow, heart, and other organs. In general, translocation rates are
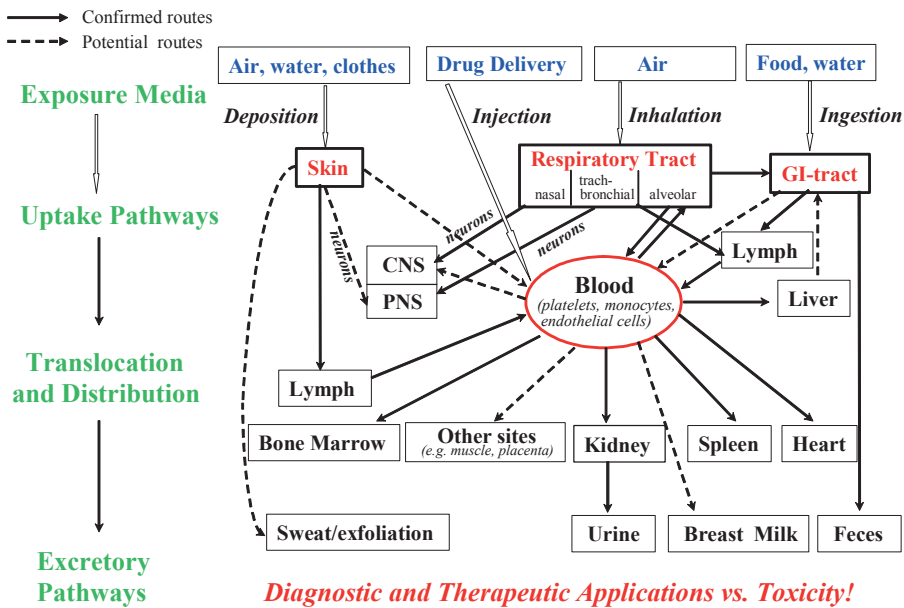

Figure 17. Biokinetics of NSP. While many uptake and translocation routes have been demonstrated, others still are hypothetical and need to be investigated. Largely unknown are translocation rates as well as accumulation and retention in critical target sites and their underlying mechanisms. These as well as potential adverse effects will be largely dependent on physicochemical characteristics of the surface and core of NSP. Both qualitative and quantitative changes in NSP biokinetics in a diseased or compromised organism need also to be considered.

largely unknown; they are probably very low but are likely to change in a compromised/diseased state (Fig. 17).

The biologic activity and biokinetics are dependent on many parameters: size, shape, chemistry, crystallinity, surface properties (area, porosity, charge, surface modifications, weathering of coating), agglomeration state, biopersistence, and dose. These parameters are likely to modify responses and cell interactions, such as a greater inflammatory potential than larger particles per given mass, translocation across epithelia from portal of entry to other organs, translocation along axons and dendrites of neurons, induction of oxidative stress, pro-oxidant, and antioxidant activity of NPs in environmentally relevant species, binding to proteins and receptors, and localization in mitochondria.

The principles of cellular and organismal interactions discussed in this article should be applicable for both ambient UFPs and NPs, even if the latter are coated with a biocompatible material. Knowledge about the biopersistence of this coating is as essential as is knowledge about the bioavailability of the core material that could have intrinsic toxic properties, for example, semiconductor metal compounds in sub-10 nm quantum dots consisting of cadmium and lead compounds. The very small size of these materials makes them available to the same translocation processes described for polydisperse UFPs, possibly even in a more efficient way because of their uniform size. When studying biologic/toxicologic effects, new processes of interactions with subcellular structures (e.g., microtubuli, mitochondria) will likely be discovered. The diversity of engineered nanomaterials – whether in medicine or in industrial applications – and of the potential effects represents major challenges and research needs for nanotoxicology, including also the need for assessing human exposure during manufacture and use. Identifying a hazard of a specific nanomaterials through appropriate toxicity testing, including dose–response assessment, by having some knowledge about the human exposure to this nanomaterials, will allow to characterize an associated risk, and if necessary to establish risk management measures (Fig. 18). For the risk assessment process, it is important to also consider the more susceptible parts of the population, the very young, the elderly and those with a compromised organ system, a lesson learned from epidemiological studies of the effects of fine particulate air pollution (Pope and Dockery, 2006). The goal to exploit positive aspects of engineered nanomaterials and avoid potential toxic effects can best be achieved through a multidisciplinary team effort involving researchers in toxicology, materials science, medicine, molecular biology, bioinformatics, and their subspecialties.
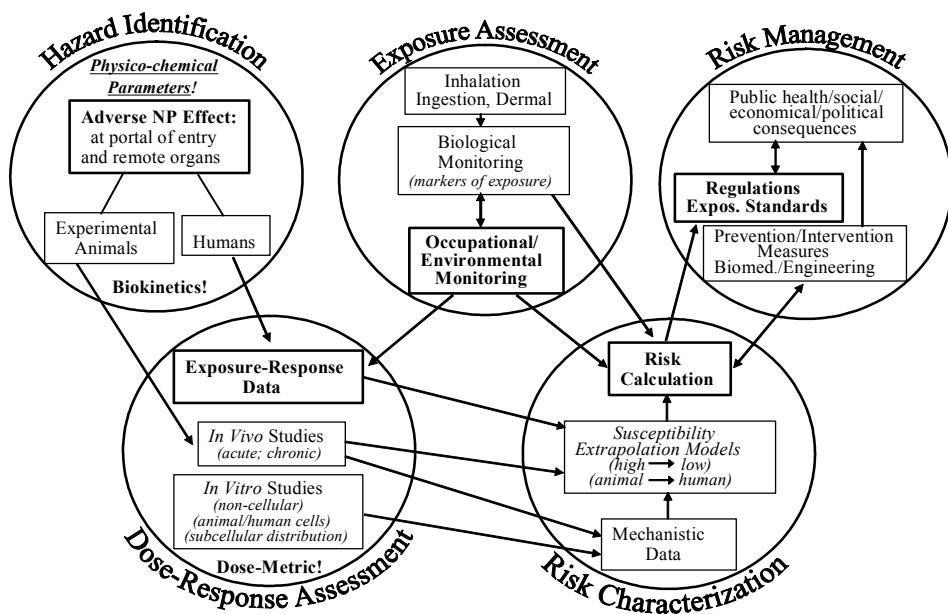
Figure 18. Risk assessment (NRC 1983) and risk management paradigm for engineered nanoparticles (NP). The four steps of risk assessment require answers to the questions: Do NP have adverse effects? What are the dose–response relationships? What are occupational/ environmental levels in different media? What is the calculated risk? Once a risk is determined, risk management decision can be established, including exposure standards and regulations and efforts for effective risk communication. (Modified from Oberdörster et al. 2004.)

Acknowledgment

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